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On the Histology of the Vegetative Organs of Brasenia peltata, Pursh.*

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Plates LXXVII and LXXVIII.

In October, 1884, while examining the structure of the stem and petiole of *Brasenia peltata*, I found some peculiar hairs projecting into the wide intercellular air-passages, which were entirely different from the so-called 'internal hairs' of *Nymphæa*, *Nuphar* and *Limnanthemum*.†

About two years later, similar hairs were noticed by J. F. A. Mellink in some petioles of *Nymphæa alba* collected by DeVries in Amsterdam, and described in Botanische Zeitung, Nov. 5. 1886.

Since that time I have carefully examined these structures, and, finding other remarkable peculiarities about this common water-plant, I thought them of sufficient interest to be placed on record.

What is described in the manuals as the creeping rootstock, is really a system of runners that proceed from the rhizoma proper. This must be rarely fully developed, for although I repeatedly searched for it carefully, I could find only few specimens. They were only from two to four cm. long and up to one cm. thick, and had very short internodes, so that they appeared covered with the scars caused by the falling off of petioles and stems of former seasons. From the rootstock proper grow the leaves and in their axils the stems. Many of the latter develop into stout runners, creeping on the surface of the ground, with internodes

* Read at the meeting of the A. A. A. S., at New York, Aug. 15 and 16, 1887, to which a few more recent observations have been added.

† I briefly described them at the October meeting of the Torrey Botanical Club (as appears from the report of that meeting in the BULLETIN), and exhibited some slides with mounted specimens. Mr. P. H. Dudley had the kindness to photograph one of the sections, showing an air-passage with some of these hairs.

up to 35 cm. long and 8 mm. thick. At the nodes the runners send out roots, usually in two lateral groups, also leaves, and vertical stems bearing leaves and flowers, besides, branches that develop into runners, forming frequently an extensively ramified system.

The secondary roots, arising at the nodes, are slender and long, often reaching 30 cm. The older branch into numerous, finally very thin rootlets provided with root hairs. At the tip of every rootlet there is a sheath or case which looks exactly like the finger of a glove. (Fig. 1.) When placed in glycerin the cells of the root contract somewhat, and the sheath becomes more plainly visible. It consists of a single layer of elongated, oblong cells, forming a distinct, firm membrane with an unbroken, smooth rim, and extends on the main roots for a distance of over 2 mm. above their growing ends. The sheath is fully developed at the tip of the forming rootlet, before it breaks through the epidermal layer, which is raised, then bursts, and persists at the base of the root, similar to the coleorrhiza of grass-seedlings.

Although there can be no doubt that this sheath has to perform the same office as an ordinary root-cap, viz., to protect the tender meristematic tissues of the root-end, its peculiar structure tempts me to ascribe to it an additional function. The upward strain exerted upon the stems and the entire root-system of our plant by the agitated surface of the water and the buoyancy of the plant itself with its floating leaves must be very great indeed. It seems to me that the innumerable sheaths at the ends of the roots and their branches must assist the plant a great deal in resisting that upward pull by acting somewhat like anchors. They are easily pushed forward and downward into the loose muddy soil along with the growing root-ends, but when pulled upward they will evidently resist the strain in a most effective manner. The fact that other aquatic plants have very similar root-caps seems to corroborate this view.

Otherwise the roots present no peculiarities. There is a central, very thin plerom surrounded by thin-walled endodermis cells; the other root-tissues are very loose, with large intercellular canals, and a thin epidermis.

What is most remarkable in the structure of the fully devel-

oped stem is the total absence of lignified tissue in all the internodes. It is true that in all aquatic phanerogams there is a considerable reduction of the vascular tissue, but in related plants, such as *Nymphæa* and *Nuphar*, we still find some very distinct tracheal ducts, besides the numerous "internal hairs," the thick walls of which are decidedly lignified. As in most other aquatic plants, a large portion of the stem is occupied by a number of very large intercellular canals, (Fig. 2, ic), which, on the cross section, have an ovate, oval, or sometimes circular outline. They are separated by partitions consisting usually of one layer of cells only. The cortical portion is made up of three or four rows of more densely arranged, large parenchyma cells, and the epidermis is of one layer of cells with a well-developed cuticle. Many of the last-named cells bear peculiar hairs, to be described hereafter.

The central portion of the stem is invariably occupied by two fibro-vascular or mestom bundles (Fig. 2, m). (As there are no vessels nor fibres proper in these bundles, the new term "mestom" is doubly preferable to the old "fibro-vascular bundle"). The two mestom bundles are separated by parenchymatous tissue and groups of intercellular canals. Each is composed of two large bundles of sieve-tubes with their accompanying cells, (Fig. 2, l). The sieve-tubes are very wide, with finely perforated, oblique septa. The sieve or "leptom" bundles have, on the cross-section, an ovate outline, the pointed ends being directed towards a large circular intercellular canal, that takes the place of the vascular or "hadrom" element of the mestom, (Fig. 2, h). This canal, as well as the leptom bundles, are strengthened at their circumference by a row of thick-walled cells. The whole mestom bundle is surrounded by an endodermis which plainly shows the "black dots" on the radial walls. At any height of a given internode the cross-sections are alike: nowhere could be found any anastomosis between the two separate bundles of an internode, as is the case in related plants.

When we arrive at the immediate proximity of the node, the large intercellular canals come to an end. The peripheral ones are closed with a spongy tissue of stellate cells, through the meshes of which the canals below the node are in open communication with those of the next internode. In the central intercellular canal

of the mestom we see, as we approach the node (Fig. 3, h), the free, pointed ends of some spiral tracheæ projecting from the sides and from above into the open space of the canal (Fig. 3, sd). The higher we proceed the more crowded with such free ends of tracheæ do we find the interior of the canal, until, at last, we have in place of it, a solid bundle of spiral ducts. We now trace, on longitudinal sections, the course of this bundle through the node, and observe how it divides into anastomosing branches, leading to the leaf of the node, to the axillary buds, eventually to the secondary roots, and to the succeeding internode. Above the node the tracheæ terminate as abruptly as they started below it; their free, tapering ends crowd into the end of the canal that belongs to the mestom of the next internode, forming a concave bottom for it just as their opposite ends form a concave roof for the canal in the next lower internode. In the meristematic tissue of the youngest internode we can trace an exceedingly delicate annular vessel, which, however, very soon disappears and is replaced by the rapidly widening canal of the mestom. The leptom elements are continuous through the node.

The structure of the petiole is like that of the stem, but in place of two mestom bundles there is only one descending to the stem, being, however, in all respects like those of the stem. The connection between the homologous parts of petiole and stem is effected in exactly the same manner as just described.

The thick, oval, peltate leaf, sometimes almost 4 in. long, has, at most, twenty principal veins, converging at the center, over the petiole. The spiral vessels of all these veins empty, as it were, into the central intercellular canal of the petiole, or rather, draw the supply of water for the leaf from it, just like a number of distributing pipes that are connected with a large reservoir. The leaf has a smooth, cuticularized upper epidermis with very numerous breathing pores, about 325 on a square millimeter. In the region above the petiole, however, on a oval surface of an average diameter of 2.5 mm. there are no stomata at all.

The cells of the upper epidermis have a peculiar structure. They are two or three times as high as they are wide; in thin sections their vertical walls at first present the appearance of deeply indented wavy lines, but on closer examination we per-

ceive that this appearance is caused by very large and deep hollows in the cell walls, distributed in such a manner that the depressions in the wall of one cell correspond to elevations or projections in that of the contiguous one, and vice versa, (Fig. 4); besides, deep pits are found abundantly in the thick walls of these cells. The palisade tissue underneath the epidermis is composed of two or three, sometimes even four tiers of cylindrical, narrow cells, with numerous air-spaces between them, and containing, on their vertical walls, large chlorophyll grains. The air cavities under the breathing pores are very large and deep, on account of the great height of the epidermis and palisade layers. The arrangement of the palisade cells into distinct groups is very striking. The upper layer of each group is joined to a group of epidermis cells (which is determined by the position of the surrounding stomata or, rather, their air cavities), while the lowest tier connects with some large "collecting cell" of the spongy tissue or of the conducting bundle.

The leaf structure is greatly modified in the central zone. The absence of stomata mentioned above causes the absence of the corresponding air-cavities, and the palisade tissue is reduced to one or two layers of cells; in the very center there are no typical palisade cells at all. Besides, the epidermis cells of this zone have almost perfectly straight walls which do not bulge outward and inward. This seems to justify the assumption that the peculiar structure of the epidermis cells has something to do with the breathing process. Supposing that the undulating walls of these cells could be straightened out, or that the elevations could even be changed into depressions and reversely, by changes in the turgor or the atmospheric pressure, we could easily infer that, according to circumstances, either a powerful suction or pressure would be produced. Such pressure seems certainly to be necessary to keep all the numerous large passages in leaf, stem and root filled with air in order to resist the great pressure of the surrounding water, and to renew the same constantly, so that the chlorophyll, which is met with almost all over the plant, may do its work of assimilation.

In connection with these considerations, I might mention that such contrivances as are supposed to assist the plant organs in

resisting positive and negative radial pressure, e. g., diaphragms, "internal hairs," as in *Nymphæa, Limnanthemum*, etc., are entirely wanting in *Brasenia*. As mechanical or stereom elements are to be considered only the outer walls of the epidermis cells, the walls of the cells which bound the intercellular canals and at the circumference of the mestom, all of which are somewhat thickened in the manner of collenchyma.

Returning to the description of the leaf, I have to mention that the spongy tissue underneath the palisade cells fully deserves its name, as the spaces between the "arms" or rays of the stellate cells are very large. The cell walls enclosing these spaces are thickly covered with a granular, crystalline layer of calcium oxalate. Even the elongated cells of the conductive tissue of the fibro-vascular bundles show this calcium oxalate coating wherever they border upon the air spaces; the inner walls of the lower epidermis cells, however, are free from it. The conductive system of the leaf is well developed. On a cross-section through one of the strong radiating veins, we see that the bundle is surrounded with a starch sheath. The hadrom contains two or three annular or spiral vessels, while the leptom occupies the bulk of the mestom. Very numerous smaller veins, similar in structure, branch off from the principal ones, anastomosing with one another, and forming the typical wavy curves at the margin.

Over the middle of many of these curves I found, on the lower epidermis, groups of very small water-pores, the number in each group varying from 10 or 15 to as many as 50. (I have also lately noticed water-pores on the lower side of the leaves of our two common species of *Nuphar;* in *Limnanthemum* I had seen them long since.) The occurrence of water-pores in these plants seems to furnish additional evidence that, even in aquatic plants, the conduction of water is effected chiefly, if not exclusively, through the vascular ducts. The water-pores in *Brasenia*, being in direct communication with the finest ramifications of the tracheal system, do most likely perform the same office as the water-pores in terrestrial plants, namely: the rapid removal of an excess of water from the conductive tissue—the lower epidermis is made up of flat cells of irregular, deeply sinuate outline, and is covered with numerous hairs.

The above description refers to the floating leaves. There are, however, thin, bright green, submerged leaves produced by *Brasenia*, which I noticed and reported some years ago. They grow at the base of the stem in limited number (I never found more than two or three on the same stem). In outline they resemble the floating leaves, but they are not longer than one inch, usually much smaller. Their blade is quite thin compared with that of the floating leaves, consisting of only four layers of cells. The upper and lower epidermis both have flat cells with wavy outlines. Both are, of course, destitute of breathing pores, but the lower epidermis is provided with very small water-pores at the margin, also with hairs like those on other parts of the plant. The assimilatory layer, under the upper epidermis, has oval cells, elongated parallel to the leaf surface; the chlorophyll occupies the lower and upper horizontal walls. Chlorophyll grains are also seen in the other layers, especially in the third, which is a very much reduced spongy parenchyma. The conductive tissue is likewise only poorly developed, but slender annular vessels and very narrow leptom elements can be plainly distinguished.

Of the peduncle I will only mention that it possesses three mestom bundles, each of which, however, has only one leptom group and only one intercellular (hadrom) canal, all of the latter facing the center of the peduncle.

Every collector, no doubt, has found it a rather difficult task to prepare good herbarium specimens of *Brasenia*, on account of the thick layer of mucilage that coats nearly all the parts of the plant in contact with the water, causing it to adhere to the drying paper.* As I do not know of any published investigations in reference to this mucilage, permit me to state my observations as to its origin and nature. If we examine the epidermis of parts which are in contact with the water, we find it thickly beset with hairs. I counted as many as 560 on one square mm. of leaf surface. On the older parts of the rhizoma and the stems the hairs occur neither in such abundance nor are they as active as on all the younger organs, especially the growing apex of the

* To obviate this difficulty I placed the fresh specimens between sheets of muslin, from which they can be detached much more easily when dry than from paper.

axis, and on the lower surface of the leaves. They are absent altogether on the upper leaf surface, and also on the lower surface within a narrow zone bounded by the leaf margin and the anastomosing curves of the marginal veins; most likely, in order not to obstruct the water-pores situated there. The cells bearing the hairs are smaller than the surrounding epidermis cells, and usually wider toward the surface. In the stem and petiole they are nearly square, and each is wedged in between four of the elongated epidermis cells, while on the leaf their cross-section parallel to the surface is nearly circular, and each is bounded by from 5 to 8 of the surrounding wavy cells. Each hair has a very short pedicel, formed of two flat and low circular cells (Figs. 10-14). In one single case I observed a pedicel consisting of three cells. In the much thickened outer wall of the epidermis cell there is a wide canal tapering toward the first pedicel cell, so that the latter is separated from the former only by a small, thin, circular septum. The walls separating the pedicel cells are very finely perforated membranes, resembling the plates of sieve-tubes. The vertical walls of both flat cells are cutinized: chloriodide of zinc will show this very plainly. Concentrated sulphuric acid will dissolve the cellulose elements and leave the cutinized portions of these walls as well defined rings on the cuticle of the epidermis of which they are a continuation.

The hairs themselves are all unicellular, but vary very much in size and shape (Figures on Plate lxxviii). Their most common form is that of a slender cylinder with a tapering blunt end, and their ordinary length is from .1 to .2 mm., the width uniformly about .04 mm. On young, growing parts, especially, we find very slender, thinner hairs, that are often as long as one millimeter. The typical form of the hairs is very often greatly modified; some are club-shaped, globular, scythe or sickle-shaped; many divide, either directly at the base, or more frequently above, into two equal or unequal branches; others again, particularly on the leaf-blade, expand horizontally in the upper portion, either to the right or left, with or without a stalk—in the latter case the hair assuming the shape of a T. By these differently shaped hairs, the mucilage peculiar to *Brasenia* is produced. We cannot fail to discover, especially on the younger

parts, some hairs which are surrounded by an inflated, bladder-like sac, often three or four times as wide, and twice as long as the hair, very frequently much longer. The bladder commences at the line of insertion of the hair on its pedicel (Figs. 12-14); in fact, it is a film of cuticle continuous with the cuticle of the epidermis, and raised from the cellulose body of the hair by a mass of mucilage accumulating under it. Chloriodide of zinc will show that the wall of the hair inside the bladder really consists of cellulose, while the membrane of the sac is stained bright yellow. This reaction will take place still more readily if applied after short treatment with sulphuric or nitric acid. While examining the effect of these reagents, there will be noticed a great many hairs, the sacs of which have burst at the top, after having elongated often to five times the length of the hairs (Figs. 13, 17). In other cases the entire sac has been torn off and carried away by the increasing mass of mucilage, which is still kept together by the thin but firm membrane of the sac.

The mucilage itself is a viscid, coherent and very slippery substance. It is colorless but highly refractive, so that it can easily be noticed around thin sections examined in water. It coagulates in alcohol, boiling water will not dissolve it, but potassic hydrate, sulphuric and nitric acids soon destroy it. Chloriodide of zinc gives it a faint grayish color; potassic iodide and sulphuric acid color it yellow. Nigrosin, an important reagent for vegetable mucilage, stains it blue; corallin slightly red, and osmic acid very light brown. Hanstein's aniline and methylene blue color it red and blue, respectively. But these aniline dyes have a still more intense effect on the numerous small and large fragments of the sacs of cuticle mixed with the mucilage, and also upon the countless hosts of a peculiar kind of *Bacterium*, of the *Bacillus* form, that are to be seen in every particle of mucilage. We might even be led to consider this substance as the product of some zoöglœa form of *Bacterium*, if we had not watched the process by which it is formed.

Returning to the examination of the hairs that secrete the mucilage, we select one of the very youngest, involute leaf buds, a transverse section of which will exhibit all the stages of development of the hairs. Nearest to the margin of the leaf we discover

that some of the epidermis cells are slightly higher than the others; some bulge out considerably above the level of the leaf surface (Fig. 8). Soon this protrusion is separated from the mother cell by a cross-partition (Fig. 9), and is afterwards raised still higher by the intercalation of two (rarely one or three) pedicel cells (Fig. 10). At the earliest stages all the cells are filled with turbid, granular protoplasm, which, as the hair increases in size, is replaced, or rather, crowded to the wall of the cell by a yellowish white mass of mucilage, which makes its appearance in the interior of the cell (Fig. 10).

This coherent, bulky, homogeneous, slightly translucent substance keeps increasing with the growth of the hair, closely surrounded by the layer of protoplasm, in which currents become plainly visible. As the hair gradually elongates, either horizontally or vertically, globular vacuoles are formed at various places in the cell. They finally merge into one or two, sometimes three or four, each occupying the entire width of the hair, and confining the mass or masses of mucilage between them (Figs. 11, 12, 14). Besides the parietal layer, thin strands and plates of very actively streaming plasma may be noticed, which extend all over the cell, carrying with them smaller and larger globular microsomata (Figs. 11, 12). Streaming protoplasm is also seen in the narrow space between the cell wall and the mucilage. In hairs in which the plasma is at rest, the microsomes at first create the impression of being imbedded in the mucilage; but even then exact focussing will destroy this illusion. The nucleus is found only with difficulty.

In some of the hairs, at various points of the surface, a slight swelling or bulging of the outermost layer of the epidermis may be noticed; in others the swelling has extended over a considerable portion of the hair (Fig. 11), and with a great many others, a complete, closed, bubble-like sac, as described above, surrounds the entire hair (Figs. 12, 14, 16). The sac, when examined without the application of any reagents, appears filled with an almost transparent, homogeneous, mucilaginous substance. It keeps increasing in size until it reaches, in many instances, several times the length and width of the hair, and at last it bursts, usually at the top.

While the inflation of the sac is progressing, the size of the mucilage masses in the hair is perceptibly diminished; in many of the ruptured sacs, however, the mucilage is still present in considerable quantities, enclosed as before by actively circulating protoplasm. But it continues to diminish, the vacuoles become larger and larger, compressing, as it were, the mucilage between their convex poles: the edges of the corresponding concavities of the mucilage mass become irregular and jagged (Fig. 15), and finally, in older hairs, the mucilage has disappeared altogether. The plasma has now ceased to live, and appears in irregular, granular masses and particles scattered through the cell (Fig. 13).

Whether, after the bursting of the sac, a second layer of cuticle is formed and raised or not, I could not decide to my entire satisfaction. The question would not have suggested itself to me, had I not, in one single case, quite distinctly seen that, a very short time after I had observed the bursting of a sac, the collapsed film of cuticle expanded again, and that in a few minutes a complete second sac was formed within the first one, the shreds of which surrounded the upper part of the new bladder (Fig. 16). The latter persisted for six days longer, when it also burst (Fig. 17). The motion of the protoplasm had continued for three days after the formation of the second sac. A small remnant of mucilage stayed at the upper end of the hair, gradually assuming a dark brown color, and had not been secreted a week later, when the observation of the hair was given up.

With the exception of this one instance, I could not discover, among the large number of hairs examined, a single one that showed the least vestige of a ruptured sac outside of a new one. Besides, a hair which has once produced a sac seems to be deprived of a cuticular layer, for concentrated sulphuric acid or chromic acid will destroy the wall of the hair entirely, leaving only the cuticle of the pedicel cells, and the sac of the hair.

In order to learn something about the homogeneous, whitish substance in the hairs, designated as "mucilage" thus far, several reagents were applied. Glycerin, sugar and alcohol cause the plasma sac to contract in the usual manner. That portion of the parietal plasma layer which adjoins the mucilage, barely leaves

the wall, while the other part, above and below, will retire to the median line of the hair. The mucilage also contracts somewhat, but otherwise remains unchanged. If the plasmolysis caused by glycerin and sugar be interrupted at the proper time by the addition of water and the removal of the reagents, the protoplasm expands again and resumes its activity. Chloriodide of zinc produces the contraction of the plasma and colors it yellow; the included mucilage becomes pale red or pink, the wall of the hair blue, the membrane of the sac yellow, and the mucilage in the sac faintly gray, of about the same tinge as the mucilage that has escaped from the sacs. On application of iodine in potassic iodide with sulphuric acid, the mucilage takes a reddish color. The same color, only of a darker, brownish hue, is produced by concentrated sulphuric acid. Vacuoles appear in the mucilage, the plasma sac contracts and remains undissolved with the mucilage enclosed, while everything else, except the membrane of the outer sac, disappears. Diluted chromic acid also stains the mucilage red. Caustic potash dissolves the mucilage rapidly, leaving a network of protoplasm, which also soon disappears.

Osmic acid (1%) stains the mucilage masses in the hair dark blue, which soon turns into an intense black, and the plasma layer becomes very light brown. In many of the sacs the mucilage assumes a blackish color, dark enough to make them almost opaque, while in others it becomes only faintly gray, of about the same tinge as the loose mucilage outside of the sacs. Bichromate of potash in some cases causes the mucilage drops at first to expand and dissolve, and then produces a deep orange-yellow, finely granular precipitate; in other hairs the mucilage masses do not change their shape, but assume a uniformly deep orange hue. Ferric chloride and sulphate give the tannin reaction for the mucilage in the hairs, not, however, for that outside of them. Acetate of copper and acetate of iron* also show plainly the presence of tannin in the mucilage of the hairs; the latter salt stains the mucilage in the sacs and outside of them a deep orange-yellow. Strong sulphuric acid applied for a short time after the acetates of copper and of iron, imparts to the mucilage in the hairs and, in some instances in the sacs, an intense olive-

* Cf. J. W. Moll, Maandblatt voor Natuurwetenschappen, 1884.

green color. Sections mounted in water several weeks ago have retained this color thus far.

Corallin colors the mucilage a dull pink or red, and nigrosin stains all the large drops in the hairs steel-blue, the surrounding plasma faintly yellow, and the mucilage in the sacs, as well as outside of them, blue also, but its color usually disappears after it has remained for several weeks in glycerin, while the mucilage in the hairs has not thus far (for about one-half a year) lost its color. The stained mucilage masses may be forced out of the hair by pressure on the cover-glass, when it will be noted that the crushed wall of the hair invariably breaks up into a continuous spiral band. It takes considerable time to stain the mucilage with nigrosin, for as long as the cell lives the coloring matter is not admitted. I observed repeatedly that hairs placed in a nigrosin solution, so dark that it was almost opaque, stayed alive for several days, i. e., their plasma kept moving and the mucilage remained unstained.

Methylene blue, on the other hand, passes through the cell wall and the living plasma.* A drop of the concentrated solution was diluted with 5 cc. of water and some living hairs with well defined mucilage drops were placed in it. After two hours most of the mucilage masses had become distinctly blue, while the motion of the colorless plasma had ceased in nearly all the hairs. Some of these sections were then placed in water, and after a short while the plasma again became active, and after three days the mucilage in most of the hairs had lost its blue color; in some of the hairs, however, it was permanently stained, and the plasma did not recover.

Very interesting observations were made when some young hairs were treated with acetic acid. As soon as the acid was applied, the mucilage began to expand rapidly, and simultaneously a swelling of the entire hair was noticed; the mucilage crowded back the vacuoles and the strands of protoplasm towards both ends of the hair, until the entire cavity was filled with an almost transparent mass; then, sometimes at one point, sometimes at several, a slight swelling of the outer layer of the wall took place, which gradually increased until, at length, there was an entire

* Cf. W. Pfeffer, Unters. a. d. bot. Inst. zu Tübingen, II, 2. Hft.

sac formed all around the hair, in all respects similar to those formed by natural growth, with the one exception that these artificial sacs did not elongate much, but, on the contrary, usually had their upper ends adhering to the corresponding extremity of the hair. Osmic acid, nigrosin and methylene blue stained the contents of these sacs much more intensely than those of the natural ones, and the hairs themselves presented the same appearance, as to color, as their sacs.

In various parts of the plant there are very many cells filled with red-colored cell-sap. They occur either singly, or in vertical rows in the stem and petiole, or in horizontal layers in the leaf, giving the surface its red appearance. The hair-bearing cells of the epidermis also frequently contain red sap. While the surrounding parenchyma cells usually contain a great deal of large-grained starch, there is little of it, more frequently none at all, in these cells. Its place is taken up, in the cells of the youngest parts, by mucilage similar to that in the hairs. Besides, there is always the most lively circulation of the plasma to be seen in them. Reagents act on the mucilage in these cells in exactly the same manner as on that in the hairs. Sections from a very young leaf were treated with acetic acid. Along the upper epidermis, the cells of which contained mucilage drops, bubble-like excrescences make their appearance just as on the young hairs treated with the same reagent. Methylene blue causes the red coloring matter to gather in one large globular drop in the middle of the cell and stains it blue, while the plasma is still living. Plasmolysis can be started and interrupted repeatedly, just as in the hairs.

The true nature and chemical composition of the mucilaginous secretion and the contents of the hairs still remain to be investigated more closely. It seems to me, however, that the reactions described positively demonstrate the presence of large quantities of tannin in the mucilage of the hairs. Furthermore, the nigrosin and corallin tests * as well as the optical inspection entitle us to call the bulky, whitish masses in the hair "mucilage." Finally, the behavior of these masses on the application of chloriodide of zinc, of sugar and of sulphuric acid, permit us to infer that nitro-

* Cf. Strasburger, Bot. Pract. pp. 106, 129, etc.

genous matter must be present in them. We might, therefore, for the present, consider this peculiar substance as a mixture of mucilage and protoplasm, impregnated with tannin.

As to the question whether the mucilage is produced in the interior of the secreting cells, or in the outer layer of their walls, we have to refer to the statements of DeBary in his Comparative Anatomy (p. 93, Engl. Ed.) where, in treating of glands, including glandular hairs, he says: "The anatomical peculiarity of the glandular parts of the epidermis consists in the appearance in the *cell wall* of that body, which is termed the secretion of the gland, as a part sharply defined from the cellular layers. The wall grows in thickness at the glandular spot by intercalation of a layer between its outer and inner side. The intercalated mass differs in material from the cellulose and cuticular wall and is termed a secretion."

Another passage on the same page reads as follows:—"More careful investigations are necessary to answer the question as to the appearance and origin of the secretion. But in any case it is incorrect to imagine a "perspiration" in the sense of a passage of large optically determinable masses formed in the interior of the glandular cells through the membrane." And on page 99 the author mentions one single exceptional case known to him: "The bases of the young leaves of *Osmunda* are covered with a rich amorphous mucilage. This originates from long septate hairs with large bead-like cells, each of which in the stages of development observed, is completly filled with a mass of mucilage. The origin of the latter remains to be investigated." *

I venture to suggest that the hairs of *Brasenia* may form another exception. In the first place we do observe "large optically determinable masses" in the interior of the hair which are similar to the secreted masses, diminishing as the secretion—in the sac—increases, and which finally disappear. Moreover we have seen that by destroying the restrictive power of the enclosing

* Since this paper was read, No. 1, Vol. I, of the Annals of Botany, has reached us, in which W. Gardiner and Tokutaro Ito discuss the structure of the secretory cells of *Osmunda regalis*, L., and *Blechnum occidentale*, L. According to their investigations, "the cell-contents usually escape by means of a small localized rupture of the wall" (p. 40), or "the whole wall through disorganization breaks down on all sides and the swollen drops quietly escape" (p. 41).

protoplasm, we can cause the rapid passage of those masses through the cellulose layer of the cell wall; for we cannot well believe that in the very short time during which this passage is completed, a transformation of a portion of the cell wall into mucilage has taken place. As another proof for the direct passage of the mucilage through the wall must be considered the absence of any swelling or striation of the cellulose layer after the rupture of the sac and while the mucilage in the hair continues to diminish and disappear. We may, therefore, assume that the increasing turgor inside of the hair forces the mucilage through the surrounding protoplasm and through the cellulose layer of the wall.

If, finally, we ask ourselves of what possible use the mucilage might be to *Brasenia*, we can only suppose that it must serve the plant as a protection against the attacks of water animals which are prevented by its slippery and yielding, but at the same time firm consistency, to crawl on its surface and to eat the tender growing parts. Moreover, the numerous kinds of larger water Algæ cannot attach themselves to the growing stems and buds, although, as mentioned before, Bacteria and also Diatoms seem to thrive in the mucilage.

In the intercellular air-canals we often meet with projecting hair-like structures. Sometimes they are simply slight protuberances from the cells lining the canal, but usually they are large outgrowths of those cells, of a cylindrical, sac-like shape, or they are inflated and widening from the base toward the rounded end (Figs. 5, 6, 7). The interior is most commonly continuous, but occasionally one to three cross-partitions may be noticed. These hairs are often found in groups of three or four, seldom singly (Fig. 7), but most frequently occurring in large numbers, all around the canal, pressing against one another and, at some points, effectually closing the entire cavity. For a considerable but varying distance from such a point many cells bordering upon the canal send such outgrowths into the open space, diminishing in number as the distance increases, and the interstices between them are usually filled with mucilage (Fig. 5).

A lining of mucilage of varying thickness is also found in such parts of the canal where there are only a few hairs or none at all (Fig. 5, mu, upper canal). The lining does not always

extend all around the canal, but often covers only patches of its wall; it frequently passes over some of the hairs, covering and enclosing them entirely. In cross-sections the outer edge of the lining is firm and smooth, assuming a distinct yellow color in chloriodide of zinc, or in potassic iodide and sulphuric acid: it is, in fact, a thin layer of cuticle raised in a coherent film from the wall of the canal by the mucilage forming under it. Wherever the mucilage occurs calcium oxalate is nearly always found in abundance; not, however, inside the cells or in separate receptacles, but imbedded in the mass of the mucilage in the form of crystals, usually octahedral, which are frequently of extraordinary size and beauty. Quite often large crystalline conglomerates cling to the sides of the canals, which are always coated besides with a dense crystalline layer of this salt.

The internal hairs have a thin membrane, and most of them bear on their surface numerous bubble-like excrescences, which sometimes attain considerable size (Fig. 6). Some rest on the hair on a broad circular base, others barely touch it at one point (Fig. 6). In the former case, when their elevation is very slight, an exceedingly thin membrane, the continuation of the outermost layer of the wall of the hair, seems to cover them. This may be noticed after treatment with nitric acid and subsequent application of chloriodide of zinc. I was unable to discover, with any degree of certainty, the least trace of a membrane around the globular bubbles. Other bubbles of exactly the same appearance and structure are frequently found on the sides of the canals, clinging to the walls of cells that have not grown into hairs.

Mellink* noticed "small hemispherical,† or more rarely pediceled bubble-like elevations" on the hairs of *Nymphæa*, and reaches the conclusion that the cuticle of the hair is thickened in some places so as to cause them. The bubbles on the hairs of *Brasenia* are not stained by chloriodide of zinc, potassic iodide, or nitric acid‡ (while the cuticle of the epidermis and the suberized parts of the endodermis react beautifully); they are evidently

* l. c.

† "Halbkreisförmige," which I suppose ought to read "halbkugelförmige."

‡ Mellink does not report the application of reagents to prove that the bubbles are really a thickening of the cuticle, but simply says that they are not hollow, because acetic acid-rosanilin will stain them uniformly red.

drops of mucilage. I frequently saw two or more of them flowing together or clinging to one another like drops of any viscid, semi-fluid substance. In some instances it can be demonstrated that the mass of mucilage filling the space between some hairs which are not in close contact, is formed of these drops, for not having perfectly coalesced they can be made out individually. How these mucilage drops are formed remains to be investigated; the internal hairs do not contain any mucilage masses as the external ones do. In many of them active protoplasmic currents and large nuclei may be observed, and others contain a great deal of starch (Fig. 7). These hairs are sure to be found in such intercellular canals as, by some agency or other, have been injured, and it is evident (as Mellink has pointed out in reference to *Nymphæa alba*) that the formation of the hairs is an effort of the plant, and in most cases a very effectual one, to repair the damage by closing up the canal. The wounds healed in this way may be caused by various mechanical forces. In several stems were found egg or larva cases suspended in the intercellular canals nearest to the surface of the stem. The animal had punctured the superficial layer of cells and deposited its eggs inside in longitudinal rows, which were plainly marked on the outside by distinct dots. These cases or sacs were obliquely suspended in the air-passage and connected with the outer world by a short narrow canal. The plant had promptly surrounded the intruder by numerous hairs of the kind described. It is not improbable that the larvæ when they leave their temporary abode, cause at least some of the wounds found on the stems.

Internal hairs are often met with, as stated above, quite far from the wound. As this was also the case with *Nymphæa alba*, Mellink thinks that these outgrowths are caused by a certain "irritation," proceeding from the wound and conducted through considerable distances by the protoplasm, which he assumes to be continuous through the cells of the affected tissues. Without doubting in the least the importance of the doctrine referring to the continuity of the protoplasm, I cannot help thinking that in our case the expansion of cells into an air-canal is rather vaguely explained by the "irritation" theory. Assuming, as above, the

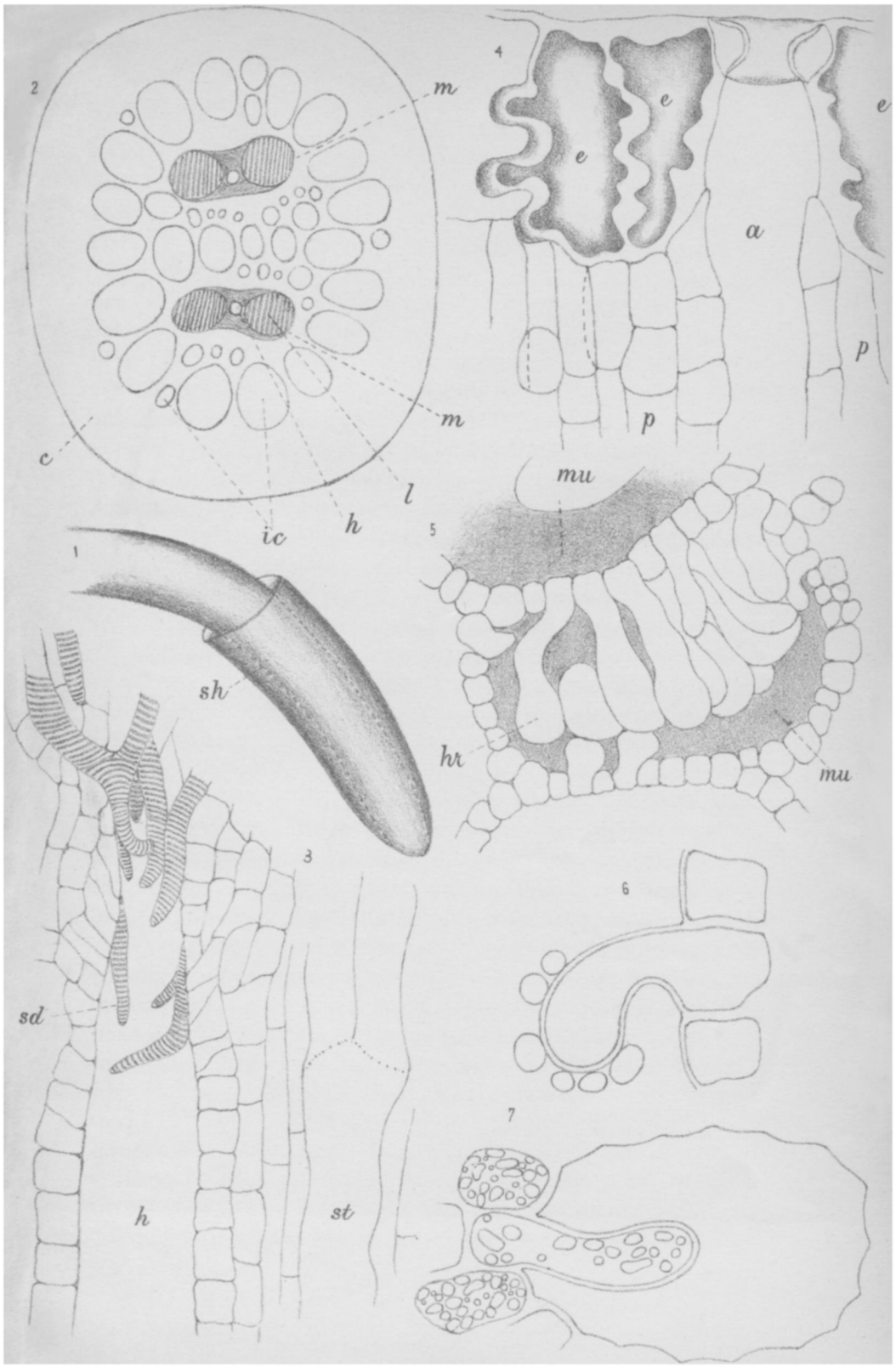
existence of a high pressure in the air-passages, we must admit that the opening of one of them by some mechanical injury would temporarily diminish that pressure. The turgor in the cells bordering upon the canal would cause them to grow in the direction of the least resistance, i. e., into the open space of the canal, until the hairs thus formed would close the opening. That the portion of the canal near the wound was most likely filled with water may be concluded from the fact that in all sections, both from fresh and alcohol material, the mucilage filling the spaces between the hairs, even at some distance above the wound, abounds in bacteria, and even sometimes diatoms, of the same kind as those that are found on the outer surface of the plant. Should these observations and conclusions prove correct, the same arguments might be applied with equal force to account for the origin of the curious formations known as tylosis, with which the hairs in question have many analogies.*

EXPLANATION OF PLATES.

PLATE LXXVII.—Fig. 1—Rootlet with enveloping sheath, sh. × 20. Fig. 2—Cross-section of stem; c, cortical portion; ic, intercellular air-canals; m, mestom; h, hadrom canal; l, leptom (The shaded part between the leptom bundles—parenchyma cells—takes up more space than the tissue it represents really does.) × 20. Fig. 3—Longitudinal tangential section of stem near node, through mestom (m, Fig. 2); h, hadrom canal; sd, spiral ducts: st, sieve-tube. × 220. Fig. 4—Cross-section of floating leaf near upper epidermis; e, epidermis cells; p, palisade cells; a, large air-cavity under stoma × 500. Fig. 5—Cross-section of intercellular air-canal in petiole, almost filled with internal hairs, hr, and mucilage, mu; one of the canals bordering on it is lined with a thick layer of mucilage, mu. × 130. Fig. 6—Internal hair beset with mucilage drops. × 430. Fig. 7—Internal hair containing starch. × 220.

PLATE LXXVIII.—Fig. 8—Epidermis cell beginning to produce a hair; e, adjoining epidermis cells. × 600. Fig. 9—the same, after the formation of a cross-partition. × 600. Fig. 10—Young hair; pc, pedicel cells; pr, protoplasma layer; mu, mucilage. × 600. Fig. 11—Hair with incipient mucilage sac, ms; mu, mucilage; pr, protoplasm (in circulation); pc, pedicel cells. × 500. Fig. 12—Hair with mucilage sac × 500. Fig. 13—Hair with sac burst; mucilage has disappeared, and granular dead plasma remnants are left. × 500. Fig. 14—Hair with two large mucilage drops. × 230. Fig. 15—Portion of hair with mucilage much reduced; the dotted curves are to indicate the double-concave shape of the drop (cf. text). × 500. Fig. 16—Hair with remnants of first sac and entire new one. × 230. Fig. 17—Same hair after bursting of second sac (cf. text, p. 39). × 230.

* Cf. Mellink, l. c.



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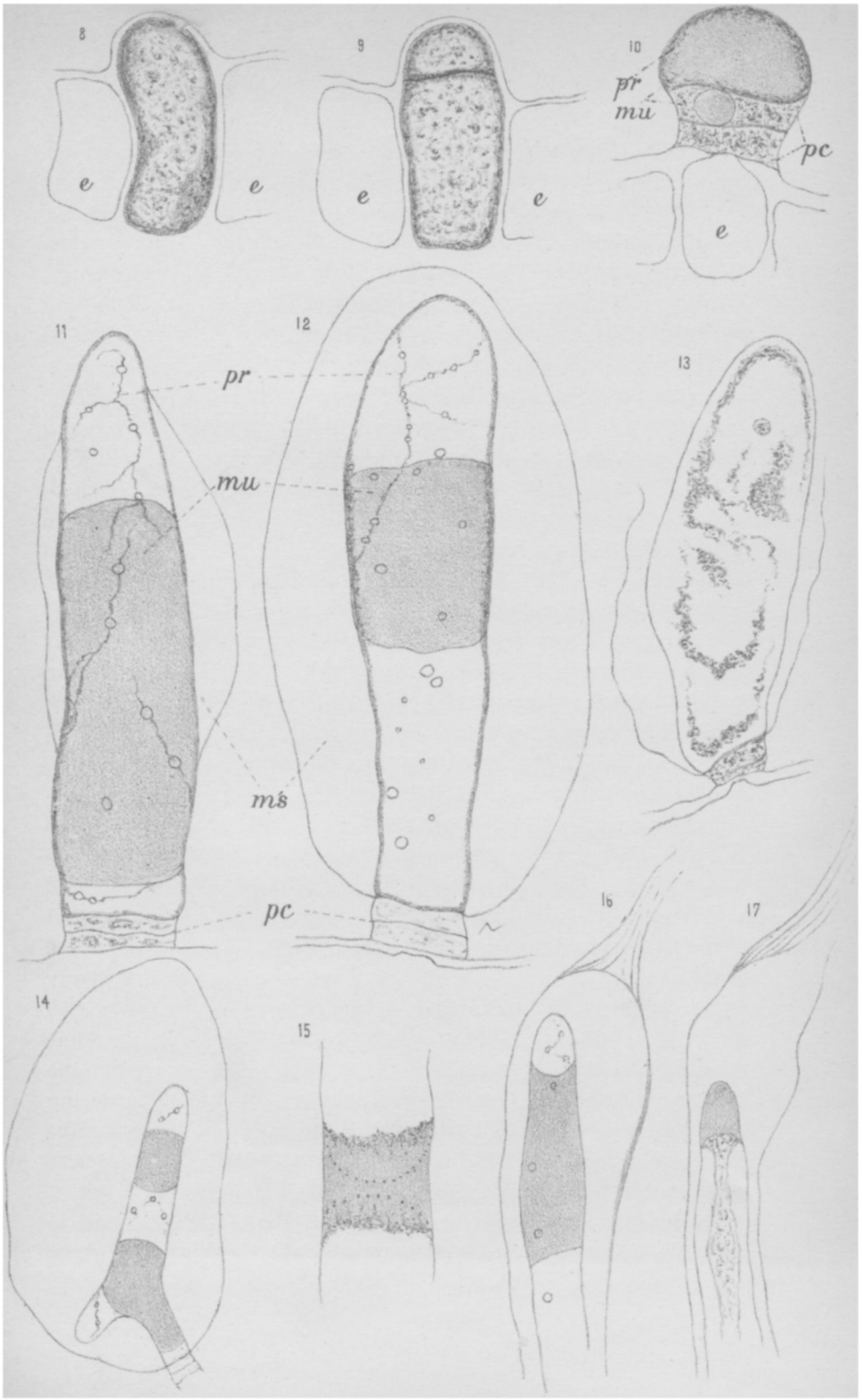
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